PROJECT TITLE

Pharmacogenomic and circulating biomarkers for predicting toxicity or suboptimal benefit from small molecule kinase inhibitor therapy

PRINCIPAL INVESTIGATOR
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SCIENTIFIC SUMMARY

Background, Rationale, and Importance:
Kinase inhibitors are an important class of targeted therapies which have proven to be highly effective for the treatment of various cancers, including lung, kidney, GI, and breast cancers. However, kinase inhibitors are well known for unpredictable and sometimes dose-limiting adverse effects. Kinase inhibitors are mostly metabolized by a drug metabolizing enzyme known as CYP3A4, currently thought to be the most important drug metabolizing enzyme in humans, due to its role in the metabolism of nearly 50% of all prescribed medications. Interestingly, the extent of interpatient variation in CYP3A4 activity is nearly 400-fold. Thus, it is not surprising that many patients experience severe adverse side effects during kinase inhibitor therapy. Expression of CYP3A4 is regulated by a xenobiotic sensing nuclear receptor known as pregnane X receptor (PXR). Activation of PXR can markedly increase expression of CYP3A4. Currently, there is no predictive biomarker, whether genomic or endogenous that could aid treating oncologists with predicting toxicity or suboptimal response to kinase inhibitor therapy.

In Aim 1, we will identify patient-specific or rare CYP3A4 and PXR single nucleotide variations (SNVs) using NextGen Sequencing (NGS) in a large cohort of estrogen receptor breast cancer patients on tamoxifen therapy.

Methods:
We will carry out whole exome NGS in a retrospective cohort of breast cancer patients (N=500) to identify common as well as rare genetic variation in CYP3A4 and PXR.

In Aim 2, we will utilize CRISPR-Cas9 technology for the creation of patient-specific or rare CYP3A4 and PXR variants in HepG2 cells and carry out high throughput in vitro CYP3A4 enzyme activity and expression profiling.

Methods:
We will utilize CRISPR gRNAs and donor DNA sequences for CYP3A4 and PXR variants of interest, where enzyme activity will be determined using ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) and expression assessed using a high throughput CYP3A4 report assay.

In Aim3, we will measure plasma 4ß-hydroxycholesterol and CYP3A4 mRNA levels from our cohort of breast cancer patients on tamoxifen therapy as endogenous biomarkers of CYP3A4 activity.

Methods:
UHPLC-MS/MS will be used to measure 4ß -hydroxycholesterol levels and qPCR to measure CYP3A4 mRNA in our biobanked plasma samples of breast cancer patients on tamoxifen.

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SCIENTIFIC SUMMARY (CONTINUED)

In Aim 4, we will create a predictive biomarker-based model of CYP3A4 activity and compare the performance of the model in a cohort of breast cancer patients who are on cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor therapy.

Methods:
In this proof of principle aim, we will perform Sanger sequencing and measure circulating CYP3A4 mRNA as well as 4ß-hydroxycholesterol plasma level in a prospective cohort of patients (N=100) who are on a CDK4/6 inhibitor (abemaciclib, ribociclib, or palbociclib) to assess whether the predicted CYP3A4 activity predicts measured CDK 4/6 inhibitor plasma level.

Expected Outcome:
Our innovative approach has significant potential to reduce adverse effects while ensuring therapeutic benefit from this important class of anticancer medications. The resultant findings will then set the stage for clinical validation of our proposed individualized vs standard approach in future clinical trials.